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Hessian fly resistance genes *H16* and *H17* are mapped to a resistance gene cluster in the distal region of chromosome 1AS in wheat

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Abstract Hessian fly [*Mayetiola destructor* (Say)] is one of the major insect pests of wheat (*Triticum aestivum* L.) worldwide. Hessian fly (Hf)-resistance genes *H16* and *H17* were reported to condition resistance to Hf biotype L that is prevalent in many wheat-growing areas of eastern USA, and both of them were previously assigned to wheat chromosome 5A by their linkage to *H9*. The objectives in this study were to (1) map *H16* and *H17* independent of their linkage with *H9* and (2) identify DNA markers that co-segregate with *H16* or *H17*, and that are useful for selection of these genes in segregating populations and to combine these genes with other Hf-resistance genes in wheat cultivars. Contrary to previously reported locations, *H16* and *H17* did not show linkage with the molecular markers on chromosome 5A. Instead, both of them are linked with the molecular markers on the short arm of chromosome 1A (1AS). The simple sequence repeat (SSR) marker *Xpsp2999* and EST-derived SSR (eSSR) marker *Xwem6b* are two flanking markers that are linked to *H16* at genetic distances of 3.7 and 5.5 cM, respectively. Similarly, *H17* is located between markers *Xpsp2999* and *Xwem6b* at genetic distances of 6.2 and 5.1 cM, respectively. Five other SSR and eSSR markers including *Xcfa2153*, *Xbarc263*, *Xwem3a*, *Xwmc329*, and *Xwmc24* were also linked to *H16* and *H17* at close genetic distances. These closely linked molecular markers should be useful for pyramiding *H16* and *H17* with other Hessian fly resistance genes in a single wheat genotype. In addition, using Chinese Spring deletion line bin mapping we positioned all of the linked markers and the Hf-resistance genes (*H16* and *H17*) to the distal 14% of chromosome 1AS, where Hf-resistance genes *H9*, *H10*, and *H11* are located. Our results together with previous studies suggest that Hf-resistance genes *H9*, *H10*, *H11*, *H16*, and *H17* along with the pathogen resistance genes *Pm3* and *Lr10* appear to occupy a resistance gene cluster in the distal region of chromosome 1AS in wheat.

Keywords *Triticum aestivum* · Microsatellite · eSSR · Gene mapping · Hessian fly resistance · Marker-assisted selection (MAS)

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Introduction

Genes in wheat that confer resistance to the Hessian fly (Hf) provide the most efficient and economical means of crop protection against this damaging insect

(Berzonsky et al. 2003). Currently, at least 32 Hf-resistance genes have been identified in wheat and its wild relatives, and these resistance genes have been designated in a series from *H1* to *H32* (Delibes et al. 1997; Ratcliffe and Hatchett 1997; McIntosh et al. 2003; Williams et al. 2003; Martín-Sánchez et al. 2003; Sardesai et al. 2005; Liu et al. 2005a, b, c; Wang et al. 2006; Zhao et al. 2006). Many of these genes, including *H6*, *H9–H11*, *H14–H20*, *H28*, *H29* and *H31*, were identified in tetraploid durum wheat, *Triticum turgidum* (AABB, $2n = 4x = 28$) ssp. *Durum* Desf., and some of these genes, including *H13*, *H22*, *H23*, *H24*, *H26*, and *H32*, originated from *Aegilops tauschii* (DD, $2n = 2x = 14$) Coss. Gene *H6* was located on chromosome 5A by monosomic analysis (Gallun and Patterson 1977). Genes *H3* and *H9* were shown by segregation analysis to be linked to *H6*, and *H15* was shown to be closely linked or allelic to *H9*, composing the linkage block *H3–H6–H9–H15* (Patterson and Gallun 1977; Stebbins et al. 1982; Maas et al. 1989). In addition, *H16* and *H17* were both assigned to wheat chromosome 5A, showing linkage with *H9* and/or *H10* (Obanni et al. 1988; Patterson et al. 1988; Ohm et al. 1995). Genes *H16* and *H17* confer resistance against Hessian fly biotype L, the most virulent and prevalent biotype in eastern USA.

Molecular mapping technologies provide new tools to map genes precisely on chromosomes of wheat. Ma et al. (1994) identified restriction fragment length polymorphism (RFLP) markers that are linked to *H23* and *H24* on 6D and 3DL, respectively. *H25* was mapped on 6BL with RFLP markers (Delaney et al. 1995). Williams et al. (2003) mapped *H31* on chromosome 5BS by its linkage to a sequence tagged site (STS) marker and an amplified fragment length polymorphism (AFLP). Recently, simple sequence repeat (SSR) or microsatellite markers have greatly facilitated Hf-resistance gene mapping. *H9*, *H10* and *H11* were mapped near the distal end of chromosome 1AS (Kong et al. 2005; Liu et al. 2005a) and not on 5A, as had been previously reported (Stebbins et al. 1980, 1982). *H13* was recently mapped on the distal region of chromosome 6DS (Liu et al. 2005c) and not 6DL, as was previously reported (Gill et al. 1987). *H26* was recently mapped on chromosome 3D, rather than 4D (Wang et al. 2006). In addition, *H22* was mapped to the short arm of chromosome 1DS (Zhao et al. 2006) and *H32* was mapped to 3DL (Sardesai et al. 2005), respectively, using SSR and other PCR-based DNA markers.

The genetic interaction between *Triticum* and *Mayetiola destructor* has been established as a gene-for-gene relationship (Gallun and Hatchett 1968; Hatchett and Gallun 1970). During compatible interactions with developing seedlings, Hf larvae establish feeding sites among the leaf sheaths near the crown at the base of the plant. Larvae induce the nutritive tissue 2–3 days after initiating their attack (Harris et al. 2006) and feed for 10–11 days (Gagné and Hatchett 1989; Harris et al. 2003; Anderson and Harris 2006). Feeding by virulent larvae not only stunts plant growth but also causes seedlings to accumulate more chloroplasts (Cartwright et al. 1959; Robinson et al. 1960). The irreversible changes in wheat tissues create a satisfactory environment to allow larval growth by delivering nutrients and protecting the larvae among the stunted leaf sheaths. Wheat seedlings without an effective R gene die or produce few seeds (Anderson and Harris 2006). During incompatible interactions, Hf larval feeding triggers activation of wheat genes in defense (Williams et al. 2002; Giovanini et al. 2006, 2007). However, the first-instar larvae do not die immediately. Rather, they continue to probe and move down the plant for 3–4 days (Gallun 1977; Grover 1995) and are unable to establish permanent feeding sites (Grover 1995). Larvae do not appear to grow on resistant host genotypes and typically die within 2–5 days after attempting to feed near the base of the plant (Harris et al. 2003; Anderson and Harris 2006; Giovanini et al. 2007). This incompatible interaction appears to have little impact on the plant because infested, resistant wheat plants exhibit relatively normal growth (Gallun et al. 1961).

Continuous evolution of virulent Hf genotypes necessitates the deployment of new resistance genes to prevent crop losses due to Hf infestation. Sequential deployment of single resistance genes has been suggested as an efficient strategy for crop protection from Hf (Cox and Hatchett 1986; Smith et al. 1999). Gould (1986) predicted that the resistance of a cultivar containing multiple genes for resistance to a single biotype of the Hf could be effective up to 20 times longer than resistance of a cultivar with a single resistance gene. The development of DNA markers that co-segregate with specific resistance genes and

that are efficient to use for plant selection, greatly enhance the feasibility of combining two or more Hf-resistance genes for deployment in wheat cultivars (Williams et al. 2003).

The objectives of this study were to: (i) map genes *H16* and *H17*, independent of their association with *H9*, and (ii) identify markers that co-segregate with *H16* or *H17* and that are useful in future MAS in wheat breeding for Hf resistance.

Materials and methods

Hessian fly stocks

Hf biotype L (virulent to resistance genes *H3*, *H5*, *H6*, and *H7H8*) is maintained by the USDA-ARS Crop Production and Pest Control Research Unit at Purdue University, in a 4°C cold room as a purified laboratory stock. Hf pupae together with infested susceptible wheat plants were stored at 4°C until Hf adults were needed.

Plant materials

The wheat (*T. aestivum* L.) parent lines used for this study consisted of cultivar Len, which is susceptible to all known biotypes of the Hf; and Hf-resistant parent lines P921682 (*H16H16*) and P921680 (*H17H17*). The corresponding Hf-resistant durum wheat (*T. turgidum*) source lines were PI 94587 for *H16* and PI 428435 for *H17*. Hf resistance gene, *H16*, was transferred to the susceptible durum wheat line D6647 by backcrossing, D6647*2/PI 94587, and testing the BC_1 progeny to Hf biotype L (Patterson et al. 1988). A resulting *H16H16* line was crossed to susceptible cultivar Newton, after which six cycles of backcrossing to Newton were carried out with selection for Hf biotype L-resistant plants after each cycle of backcrossing, followed by three generations of self-pollination and testing to biotype L to identify wheat line P921682 (*H16H16*). Similarly, PI 428435 was first backcrossed to D6647, identifying gene *H17* (Obanni et al. 1988) and resulting in a *H17H17* durum line. Gene *H17* was subsequently transferred from the *H17H17* durum line into common wheat cultivar Newton by backcrossing to Newton and selection for Hf biotype L-resistant plants after each of six cycles of backcrossing followed by three generations of self-pollination and testing to biotype L, resulting in the wheat line P921680 (*H17H17*).

Triticum aestivum wheat line Chinese Spring (CS) and CS deletion lines del1AS-1 (KSU#4510-1) and del1AS-3 (KSU#4510-3) were included for mapping in this study.

Progeny screening for Hf resistance

The Hf-resistant parental lines, P921682 and P921680, were, respectively, crossed to the susceptible *T. aestivum* cultivar Len, and the resulting F_1 plants were backcrossed to Len to produce BC_1F_1 plant populations of 113 and 103 plants, respectively, from Len*2/P921682 and Len*2/P921680. BC_1F_1 plants were self-pollinated to produce BC_1F_2 seeds. Additionally, F_2 populations were derived from the crosses Len/P921682 and Len/P921680, respectively; and F_2 plants were self-pollinated to produce F_3 seeds. Plant tissue for DNA extraction was collected from all BC_1F_1 and F_2 plants. BC_1F_2 and $F_{2:3}$ families, along with parent lines Len, P921682, P921680 and resistance source lines PI 94587 and PI 428435 were scored for resistance to Hf biotype L.

Parent lines, F_1 plants and $F_{2:3}$ families, and BC_1F_2 families, together with the resistance source lines, were seeded in soil in wooden flats that were 54 × 36 × 8 cm deep, containing 10 evenly spaced rows (Ohm et al. 1995). All Hf resistance screens were conducted as progeny tests. At least 15 seedlings of each BC_1F_2 and $F_{2:3}$ family were grown in nine of the 10 rows. One center row was divided to accommodate both resistant and susceptible parental lines of 10 seedlings each as checks. Ten seedlings of each F_1 and the two durum resistance source lines were also included in the tests.

Hf bioassays were conducted similar to that described previously (Ohm et al. 1995; Anderson and Harris 2006). Briefly, plants were infested with Hf adults at the one-leaf stage by allowing the Hf to emerge from infested wheat plants under a cheesecloth tent which covered the flats. Mating and oviposition proceeded for 24 h when all adult flies were removed. After infestation, flats were moved to growth chambers maintained at constant temperature (18 ± 1°C) with a 12-h light period.

Three weeks after infestation, $F_{2:3}$ and BC_1F_2 families as well as F_1, parents and the resistance source lines were classified as resistant, susceptible or

segregating. Susceptible plants appeared stunted, with dark green leaves, and live larvae at the base of the first leaf. Resistant plants appeared normal. For families that were segregating, the non-stunted plants were dissected to distinguish non-infested escapes from resistant plants by verifying the presence of dead red larvae at the base of the leaf sheath as proof that the seedling was infested and resistant. Hf resistance was evaluated as described by Ohm et al. (1995).

DNA isolation

Genomic DNA was isolated from seedling leaves using the CTAB method described by Saghai-Maroof et al. (1984) with minor modifications. A 1.67% CTAB extraction buffer [100 mM Tris–HCl buffer pH 8.0, 1.67 % (w/v) hexadecyltrimethylammonium bromide (CTAB), 100 mM Na_2EDTA, and 1.4 M NaCl] was used. DNA concentration was quantified on a Hoefer DyNA Quant 200 Fluorometer (Hoefer Pharmacia Biotech Inc., Dubuque, IA, USA).

Bulked segregant analysis

For bulked segregant analysis (BSA) (Michelmore et al. 1991), equivalent amounts of genomic DNA from seven resistant (all of their tested $F_{2:3}$ progeny were resistant) and seven susceptible F_2 plants (all of their tested $F_{2:3}$ progeny were susceptible) from each population derived from the crosses Len/P921682 and Len/P921680, were, respectively, pooled to form resistant and susceptible bulks as described in Kong et al. (2005). Both bulks were used along with the parents to identify markers showing polymorphisms between the four samples. These polymorphic markers were further used to analyze BC_1F_1 populations to determine linkages between SSR/eSSR markers and resistance genes *H16* and *H17*, respectively.

Microsatellite and STS marker analysis

Wheat microsatellite markers on chromosome 1A and 5A designated as either *Xgwm* for Gatersleben (Germany) wheat microsatellite (Röder et al. 1998) or *Xgdm* for Gatersleben D-genome microsatellite (Pestsova et al. 2000), or *Xwmc* for Wheat Microsatellite Consortium (Gupta et al. 2002) were tested for useful polymorphisms. Additional markers including *Xbarc* (Beltsville Agriculture Research Center), *Xksum* (Kansas State University microsatellite), *Xcnl* (Cornell University microsatellite), *Xcfa* and *Xcfd* (Pierre Sourdille microsatellite), *Xpsp* (Devos et al. 1995; Stephenson et al. 1998) on chromosome 1A were also tested on the *H16* and *H17* populations from the crosses Len/P921682 and Len/P921680, respectively. Considering that both *Pm3* and *Lr10* are located on chromosome 1AS, STS marker for *Pm3* (*STS-Pm3*, forward primer 5′ *ATGGCTAGATGCCCGTTATG* 3′ and reverse primer 5′ *AGAGCAGAGCAGTGCAACAA* 3′) and the STS marker for *Lr10* (*STS-Lr10*, forward primer 5′ *GCGCTATGCCTAACCTGAAG* 3′ and reverse primer 5′ *CTCCACATAGGCAGCACTGA* 3′) were developed based on the available sequences from the GenBank database (GenBank #AY605285 and GenBank #AY270157, respectively).

Wheat EST-derived microsatellite marker analysis

The Perl script MISA (http://www.pgrc.ipk-gatersleben.de/misa.html) was used to identify SSRs in a wheat EST database containing approximately one-half million ESTs (http://www.wheat.pw.usda.gov/cgi-bin/ace/search/wEST). Twenty-one EST-derived microsatellites (eSSRs) were chosen from the short arms of chromosomes from group 1 (Table 1) using seven consensus maps (Peng and Lapitan 2005). All of the primers were designed by Primer3 (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3) and synthesized by Integrated DNA Technologies (Coralville, IA, USA).

Polymerase chain reaction

Polymerase chain reaction (PCR) for each SSR and STS marker was performed in a Bio-Rad MyCycler™. Thermal Cycler (Hercules, CA, USA) at amplifications of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s 50°C, 52°C, 55°C, or 60°C (based on primer annealing temperature) for 40 s, and 72°C for 1 min, with a final extension at 72°C for 7 min before cooling to 4°C. Each PCR (25 µl) consisted of 40 ng of template DNA, 10 mM Tris HCl, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.25 µM of each primer and 1 unit of *Taq* DNA polymerase. The amplified PCR products were

Table 1 eSSR primers targeting short arms of wheat group 1 chromosomes applied to linkage analysis of Hf-resistance genes *H16* and *H17*

Locus[a]	Left primer	Right primer	EST accession	SSR motif	Tm[b] (°C)	Amplicon size (bp)	Chromosome location
Xwem3a	GATCTGTGACCGAGGCAGA	GCTGTGGAGGTCCAAAATGT	BG607867	(AC)7	55	77	1AS
Xwem6a	CCTGCTCTGCCATTACTTGG	TGCACCTCCATCTCCTTCTT	BF483588	(AG)12	55	145	1DS
Xwem6b	CCTGCTCTGCCATTACTTGG	TGCACCTCCATCTCCTTCTT	BF483588	(AG)12	55	150	1AS
Xwem6c	CCTGCTCTGCCATTACTTGG	TGCACCTCCATCTCCTTCTT	BF483588	(AG)12	55	165	1BS
Xwem7f	ACGGCGTGTTGAGTTTTTCT	CAACTGCAACAACAAAACAGT	BE500104	(T)10	55	328	1BS
Xwem8b	TGTGCTTCAAGCCTCAAGTG	GCTCGCACTCGAGTACACTG	BE443007	(TAC)5	55	132	1DS
Xwem9a	CACCATCACCGAGATCCAA	GGAGCTCCTCCACCTTGTC	BE494877	(CAGG)5	55	78	1BS
Xwem9b	CACCATCACCGAGATCCAA	GGAGCTCCTCCACCTTGTC	BE494877	(CAGG)5	55	115	1BS
Xwem9g	CACCATCACCGAGATCCAA	GGAGCTCCTCCACCTTGTC	BE494877	(CAGG)5	55	190	1AS
Xwem10	GAACATTTTTGCGTCCTGTG	TGGTGATCCAGAAGCCATTT	BF483804	(A)11	50	68	1DS
Xwem11c	CAGAGCAACCAGATGTTGGA	TGCACGTAGTAGTAGGCACCTC	BG314086	(ACT)5	60	300	1DS
Xwem11d	CAGAGCAACCAGATGTTGGA	TGCACGTAGTAGTAGGCACCTC	BG314086	(ACT)5	60	555	1BS
Xwem12a	CAGCAACCATTACCACCACA	GCGAAAATGATGGTTGTTGA	BE424439	(ACA)5	60	100	1DS
Xwem12b	CAGCAACCATTACCACCACA	GCGAAAATGATGGTTGTTGA	BE424439	(ACA)5	60	140	1AS
Xwem12c	CAGCAACCATTACCACCACA	GCGAAAATGATGGTTGTTGA	BE424439	(ACA)5	60	153	1BS
Xwem20d	GACACCTTCTCTTGCTCCAAA	GAAGACGTGATCAGCATGGA	BE442801	(TTG)5	50	230	1AS
Xwem20f	GACACCTTCTCTTGCTCCAAA	GAAGACGTGATCAGCATGGA	BE442801	(TTG)5	50	580	1AS
Xwem25a	CGCCTCAGAGCTCTTCACC	AAGATACGGTCCGTGTAGGAG	BG607143	(CCG)5	55	110	1BS
Xwem46a	ACGTTGTCTCCGTGTCATTG	GGTCATGGCCTCAGTCTCA	BE500430	(TCC)5	50	105	1DS
Xwem47c	CCTTCTCGACTCCCTCTTCG	CCATTGCTCGTGGACCTGT	BE426787	(AGG)5	55	600	1BS
Xwem54c	AGCCAAAGGAGCTGGAGGAC	GGCTCCGTGCTCCTCGAC	BE517937	(CCG)5	55	370	1BS

[a] Wem = Wheat EST-derived microsatellite

[b] Tm = melting temperature (°C)

fractionated on 2.0–3.0% agarose gels (based on the size difference of the polymorphism) using a mixture of 1:1 Metaphor® and Seakem® in 0.5 × TBE buffer and photographed over a UV light source (Kong et al. 2005).

Linkage analysis

Data were analyzed using the chi-square (χ^2) test to ascertain goodness of fit between the expected ratio for a single dominant gene and the observed phenotypic segregation. Linkage analysis between the SSR or eSSR markers and the Hf-resistance genes, *H16* and *H17*, was performed with the software package MAPMAKER/EXP version 3.0 (Lander et al. 1987). Map units were computed by applying the Kosambi function (Kosambi 1944). The LOD score of 3 and the maximum distance of 50 cM were used in the determination of linkages.

Results

Hessian fly response phenotyping

BC_1F_1 populations of Len/P921682 and Len/P921680 segregated, respectively, 55 resistant : 58 susceptible and 54 resistant : 49 susceptible. These numbers fit a 1:1 ratio ($\chi^2 = 0.08$, $P > 0.70$ and $\chi^2 = 0.24$, $P > 0.50$, respectively) of a single dominant gene for Hessian fly resistance in both P921682 (*H16*) and P921680 (*H17*). The tests to phenotype the two BC_1F_1 populations were definitive, all seedlings of the resistant parents P921682 (*H16H16*) and P921680 (*H17H17*), the durum donor lines PI 94587 and PI 428435, as well as F_1 seedlings derived from Len/P921682 and Len/P921680 were clearly not stunted and all plants of the susceptible parent line Len were clearly stunted.

Molecular mapping of *H16* and *H17*

Because *H16* and *H17* were previously assigned to wheat chromosome 5A by segregation analysis, 40 5A-specific SSR markers were screened for linkage validation. However, no markers on either the long arm or short arm of chromosome 5A showed linkage relationships with Hf-resistance genes *H16* and *H17* (data not shown). Previously, the linkage relationships with *H9* and/or *H10* suggested that *H16* and *H17* occupied a single linkage block (Obanni et al. 1988; Patterson et al. 1988; Ohm et al. 1995), and recently *H9* and *H10* were both placed on the distal region of wheat chromosome 1AS by molecular mapping (Kong et al. 2005; Liu et al. 2005a). Therefore, *H16* and *H17* were assumed to occupy the linkage block on chromosome 1A along with other Hf-resistance genes such as *H9* and *H10*. To test the hypothesis that *H16* and *H17* are on chromosome 1A, 62 SSR markers from chromosome 1A and 21 eSSR markers from group 1 (Table 1) were screened for potential linkage to *H16* or *H17*. As expected, no SSR markers other than a few from the short arm of 1A, and no eSSR markers other than two from 1AS showed linkage with *H16* and *H17*, which confirmed that both *H16* and *H17* are on the short arm of chromosome 1A. BC_1F_1 populations of both *H16* and *H17* were further genotyped with additional SSR and eSSR markers based on BSA. Seven out of 62 SSR and 21 eSSR markers, including Cfa2153, Psp2999, Wem6b, Barc263, Wem3a, Wmc329 and Wmc24, showed linkage with *H16* and *H17*. Recombination analysis indicated that Hf-resistance gene *H16* was flanked by *Xpsp2999* and *Xwem6b*/*Xbarc263* with map distances of 3.7 and 5.5 cM, respectively. No recombination was observed between *Xbarc263* (Fig. 1) and *Xwem6b* in the Len*2/P921682 population. The *Xcfa2153* marker locus was located distal to *H16* with 9.3 cM on chromosome 1AS. *Xwem3a*, *Xwmc329* and *Xwmc24* are proximal to *H16* at 11.1, 12.0, and 19.6 cM, respectively (Fig. 2a).

The *H16*-linked SSR and eSSR markers were also linked with *H17* on chromosome 1AS (Fig. 2b). *H17* is linked to the flanking markers *Xwem6b* and *Xpsp2999* with genetic distances of 5.1 and 6.2 cM, respectively. Marker *Xcfa2153* is distal to *H17* at 10.2 cM. Two other markers, *Xbarc263* and *Xwmc24*, are proximal to *H17* at 7.1, 17.3 cM, respectively. The eSSR marker *Xwem3a* co-segregated with *Xwmc329* and both are proximal to *H17* at 11.1 cM (Fig. 2b).

All of these seven linked markers were subsequently used to screen the resistant parent lines, P921682 and P921680, and the corresponding original durum donor lines PI 94587 and PI 428435 to determine the durum chromosomal segments transferred from the donors. A small terminal

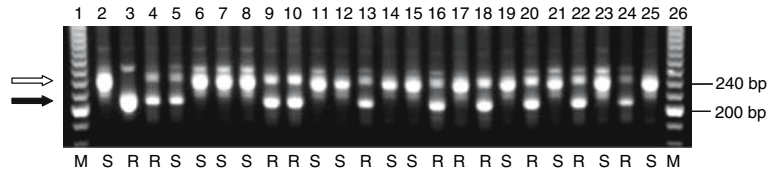

Fig. 1 DNA bands amplified from parents and 22 BC_1F_1 plants derived from P921682(*H16H16*)/Len (*h16 h16*) with microsatellite primer pair *Xbarc263*. Lane 1 = 20-bp DNA ladder, Lane 2 = Len, Lane 3 = P921682, Lanes 4–25 = BC_1F_1 plants (heterozygous resistant and homozygous susceptible are indicated by R and S, respectively). BC_1F_1 plants in lanes 5 and 17 are recombinant between *Xbarc263* and *H16*. DNA fragments slightly larger than 210 and 240 bp were amplified, respectively, from the resistant parent and resistant BC_1F_1 plants, versus the susceptible parent and susceptible BC_1F_1 plants. These bands are indicated by the *black arrow* and *white arrow* on the left, respectively

chromosomal segment in wheat line P921680 carrying *H17* was found to be most likely transferred from the donor parent PI 428435 on the basis that *H17*-linked SSR markers *Xpsp2999* and *Xcfa2153*, distal to *H17*, and *Xwem6b* and *Xbarc263*, proximal to *H17* (the black region of chromosome 1AS in Fig. 2b) were not polymorphic between the wheat line P921680 (*H17H17*) and the durum *H17* donor, PI 428435. However, polymorphisms were detected at other proximal linked markers, *Xwem3a*, *Xwmc329*, and *Xwmc24* (the white region of chromosome 1AS in Fig. 2b). Interestingly, all of the seven *H16*-linked markers showed polymorphisms between the wheat line P921682 (*H16H16*) and the durum *H16* donor, PI 94587. It is likely that a very small intercalary segment (the black region of chromosome 1AS in Fig. 2a) containing *H16* was transferred from PI 94587.

Physical mapping of the linked markers

DNA samples of CS and its derived deletion lines including del1AS-1 (FL 0.47) and del1AS3 (FL 0.86) were amplified using primer pairs of all of the *H16*- and

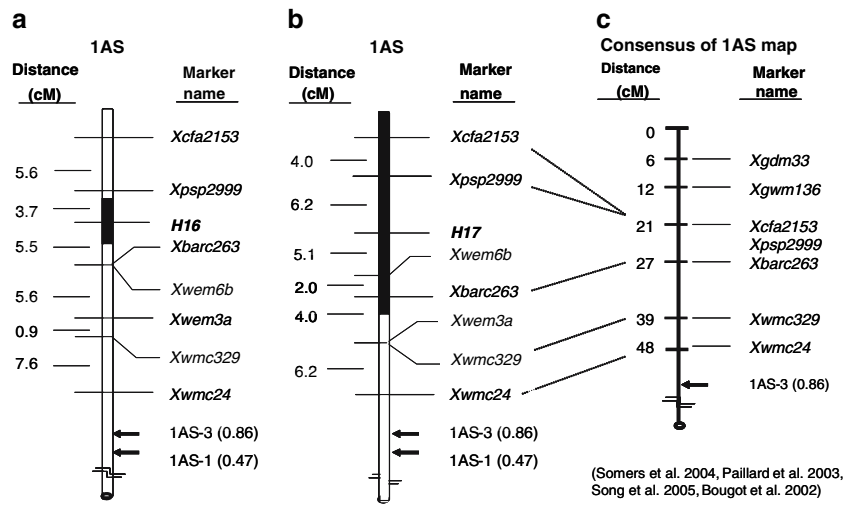

Fig. 2 Linkage map of the short arm of wheat chromosome 1A showing the genetic map location of Hf-resistance gene *H16* (**a**), *H17* (**b**), and the consensus genetic map of the chromosomal region containing the *H16*/*H17* region on 1AS (**c**). Approximate distances in centi-Morgans (cM) and molecular markers are indicated on the left and the right, respectively. The letter *X* in front of each SSR locus name indicates the basic symbol for a molecular marker with unknown function. The fraction lengths of the deletions are indicated in the parentheses, and the breakpoints of deletion bins are indicated to the right by arrows. The black regions of chromosome 1AS in (**a**) and (**b**) represent the durum donor-derived segment containing *H16* or *H17* in wheat lines P921682 and P921680, respectively. White regions of the chromosome in (**a**) and (**b**) represent the common wheat genetic backgrounds

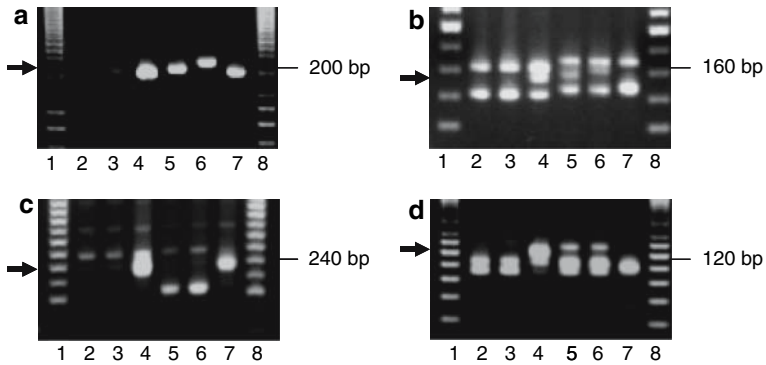

Fig. 3 DNA fragments amplified with simple-sequence-repeat primers Cfa2153 (**a**), Wem6b (**b**), Barc263 (**c**), and Wmc329 (**d**). Lane 1 = 20-bp DNA ladder, Lane 2 = CS del1AS-3 (0.86), Lane 3 = CS del1AS-1 (0.47), Lane 4 = CS, Lane 5 = P921680 (*H17H17*), Lane 6 = P921682 (*H16H16*), Lane 7 = Len, Lane 8 = 20-bp DNA ladder. Cfa2153, Wem6b, Barc263, and Wmc329 produced the specific fragments of 195, 155, 235, and 125 bp, respectively (pointed by *arrows*), only with CS

H17-linked SSR and eSSR markers including *Xcfa2153*, *Xpsp2999*, *Xwem6b*, *Xbarc263*, *Xwem3a*, *Xwmc329*, and *Xwmc24* to determine the physical locations of these linked markers. Each primer pair amplified DNA fragments of the expected size (s) from CS, but no corresponding fragments were amplified from CS del1AS-1 (FL 0.47) and CS del1AS-3 (FL 0.86) (Fig. 3a–d). This observation indicated that all of these linked markers along with Hf-resistance genes (*H16* and *H17*) are located in the distal 14% region on 1AS beyond the breakpoint of CS del1AS-3 (FL 0.86) (Figs. 2 and 3).

Discussion

Chromosome locations of Hf-resistance genes *H16* and *H17*

Recent molecular mapping analyses have provided conclusive evidence that Hf-resistance genes *H9*, *H10*, and *H11* are placed in the distal region of chromosome 1AS (Kong et al. 2005; Liu et al. 2005a). In this study, *H16* and *H17* are both mapped to the wheat chromosome 1AS by molecular techniques. Further evidence of Hf-resistance genes including *H9*, *H10*, *H11*, *H16*, and *H17* being located on chromosome 1AS came from the results of PCR amplification obtained from two CS deletion lines del1AS-1 (FL 0.47) and del1AS-3 (FL 0.86) with the primers for the closely linked SSR and eSSR markers in the current study and the previous studies (Kong et al. 2005; Liu et al. 2005a; Peng and Lapitan 2005). Hf-resistant wheat lines P921682 and P921680 carry the dominant Hf-resistance gene *H16* or *H17*, respectively, which were initially localized on chromosome 5A by segregation analysis, showing linkage to *H9* and/or *H10* (Obanni et al. 1988; Patterson et al. 1988). *H9* was previously assigned to chromosome 5A based on linkage to *H6* (Stebbins et al. 1980), and *H6* was in turn mapped to 5A by monosomic analysis (Gallun and Patterson 1977). The error in placing gene *H6* on chromosome 5A, rather than on 1A by monosomic analysis could be the result of any of a number of factors including misidentification of monosomic genetic stocks. However, mapping of *H6*, originally and erroneously on chromosome 5A, emphasizes the importance of correctly mapping the first gene when it is used subsequently as a reference point for mapping additional genes. The original location of *H6* was not validated and all subsequent locations to the same region were based on close linkage to *H6*.

Linkage analysis and physical mapping of the linked molecular markers near the *H16* and *H17* loci positioned both of these Hf-resistance genes distal to the breakpoint of del3AS-3 (FL 0.86), or the distal 14% of the short arm of wheat chromosome 1A (Fig. 2a and b). Comparative analysis of *H16*/*H17* linkage maps and the consensus genetic map of 1AS (Somers et al. 2004; Paillard et al. 2003; Song et al. 2005; Bougot et al. 2002) is illustrated in Fig. 2c. In general, the marker order near *H16*/*H17* is similar to

that of the consensus genetic map on 1AS; the linkage region was flanked by *Xgwm136* and *Xwmc24* with genetic distance about 36 cM. Since the *H16*/*H17* loci are placed in the distal bin of 1AS (del1AS-3), we attempted to generate additional eSSR markers within the telomeric region of 1AS to define the physical end of the linkage map using the EST database. Of 21 eSSRs developed from the short arm of group 1 chromosomes (1AS, 1BS, and 1DS), only two eSSR markers, *Xwem6b* and *Xwem3a*, mapped proximal to *H16*/*H17* on 1AS (Fig. 2). The genetic locations of *Xwem6b* and *Xwem3a* reported in this study are in agreement with the locations revealed by eSSR physical mapping (Peng and Lapitan 2005). Out of 15 eSSR markers derived from either 1B or 1D, none shows linkage with *H16* or *H17*, which also supports the observation that *H16* and *H17* reside on 1AS.

DNA markers have facilitated the precise mapping of alleles or homoeo-loci. In this study, the map positions of *H16* and *H17* on chromosome 1AS are very similar (Fig. 2). However, the most closely linked flanking markers, *Xpsp2999* and *Xwem6b*, showed different banding patterns between Hf-resistant parent lines P921682 (*H16*) and P921680 (*H17*). Moreover, all of the more closely linked markers showed polymorphisms between the durum donors PI 94587 (*H16*) and PI 428435 (*H17*) except for the markers *Xwem3a*, *Xwmc24*, and *Xcfa2153* flanking this linkage region of 1AS (Fig. 2). It is noteworthy that, in our previous study, SSR markers *Xgwm136* and *Xgdm33* both are tightly linked distally to Hf-resistance gene *H9* at 1.7 and 2.2 cM, respectively (Kong et al. 2005). A similar mapping result with *H9* was reported by Liu et al. (2005a). However, in this study, neither *Xgwm136* nor *Xgdm33* showed linkage with *H16* or *H17*. It is unknown if *H9* is distal or proximal to *H16*/*H17* on 1AS. The actual linkage relationship between *H16*/*H17* and *Xgwm136*/*Xgdm33* cannot be observed due to the limited polymorphisms between Hf-resistant parent lines P921682 and P921680 and susceptible parent line Len in this gene-rich region on 1AS.

Comparison of Fig. 2a and b might raise the question whether *H16* and *H17* are at the same locus. However, analysis of the testcross: D6647 (susceptible to Hf)//IN80164 (*H16H16*)/PI 428435 (*H17H17*), all three parent lines being durum wheat, showed that the genetic recombination frequency between *H16* and *H17* was 25 cM (Ohm et al. 1995).

A resistance gene cluster in the gene-rich distal region of 1AS

There are various reports that resistance genes to different pests and pathogens are linked and located in clusters observed in wheat (McIntosh et al. 1995, 2003; Adhikari et al. 2004); rice (Sardesai et al. 2002), maize (Hulbert et al. 2001), tomato (Dickinson et al. 1993), and soybean (Ashfield et al. 1998; Bachman et al. 2001). The genomic region that contains *H9*, *H10*, *H11*, *H16*, and *H17* is also particularly rich in genes for resistance against fungal pathogens. For example, *Pm3* for resistance to wheat powdery mildew (incited by *Blumeria graminis*) was also mapped with RFLP marker *BCD1434* (Hartl et al. 1993; Ma et al. 1994) and co-segregated with SSR marker *Xpsp2999* (Bougot et al. 2002) in the distal region of the short arm of chromosome 1A. At least 10 alleles (*Pm3a* to *Pm3j*) were identified at this locus (Zeller and Hsam 1998). Our previous study also confirmed that the STS marker derived from powdery mildew resistance gene *Pm3* was linked to Hf-resistance gene *H9* at a genetic distance of 4.5 cM (Kong et al. 2005). Leaf rust resistance gene *Lr10*, effective against *Puccinia triticina* Eriks, was also mapped in the same chromosomal region on 1AS (Schachermayr et al. 1997; Guyot et al. 2004). In order to determine the resistance gene order in this gene-rich region on 1AS, we designed primers for both *Pm3* and *Lr10* based on the available sequences in the database. Unfortunately, the *STS-Pm3* and *STS-Lr10* markers did not show polymorphism between the parent lines in the current study. However, because most of the SSR markers on 1AS showed linkage with *Pm3*, *Lr10*, and Hf-resistance genes including *H9*, *H10*, *H11*, *H16*, and *H17* (Kong et al. 2005; Liu et al. 2005a; Somers et al. 2004; Bougot et al. 2002; Guyot et al. 2004; Schachermayr et al. 1997), all of these resistance genes appear to compose a resistance gene cluster in the distal gene-rich region of 1AS.

The presence of multiple disease and insect resistance genes in this chromosomal region and positional cloning for both *Lr10* and *Pm3b* in bread wheat (Stein et al. 2000; Feullet et al. 2003; Yahiaoui

et al. 2004) make this genomic region much more attractive for future study including possible map-based cloning of any of the Hf-resistance genes in this region.

Marker-assisted selection being advanced by molecular technologies

Conventional wheat breeding for Hf-resistance relies on phenotypic selection through bioassays. More efficient and rapid breeding and deployment of Hf-resistant wheat varieties are critically needed because of the rapid evolution of Hf virulence. It has been postulated that the durability of resistance can be increased up to 20-fold by developing cultivars that contain multiple Hf-resistance genes (Gould 1986). Efficient pyramiding of effective Hessian fly resistance genes is possible with the aid of markers that co-segregate with the resistance genes, since expression of one gene effectively masks the presence of additional genes (Williams et al. 2003). The limiting factor for gene stacking is the lack of molecular markers specific to individual Hf-resistance genes. The results of this study are of practical significance to Hf resistance breeding. The specific and diagnostic SSR/eSSR markers closely linked to *H16* and *H17* identified in this study not only can assist wheat breeders in making parental selection but also will facilitate combining the Hf-resistance genes into elite breeding lines during cultivar development. These markers plus the other already mapped markers (Ma et al. 1994; Dweikat et al. 1997, 2002; Seo et al. 1997; Williams et al. 2003; Kong et al. 2005; Liu et al. 2005a, b, c; Sardesai et al. 2005; Wang et al. 2006; Zhao et al. 2006) will speed the development of breeding lines containing multiple resistance genes to develop broad-spectrum and durable resistance. For example, in this study, the two flanking markers, *Xpsp2999* and *Xwem6b*, are linked to *H16* at 3.7 and 5.5 cM, respectively (Fig. 2). The recombination frequencies (RF) between Hf-resistance gene *H16* and SSR markers *Xpsp2999*, *Xwem6b* are 3.6% and 5.2% (Haldane mapping function, Haldane 1919), respectively. The two RFs for *Xpsp2999* and *Xwem6b* translate into selection accuracies of 96.6% and 94.8%, respectively, if both of the markers are used separately. However, according to the product rule of the probability, the selection accuracy will increase to nearly 100% [1–(3.6% × 5.2%)] when these two flanking markers are used together.

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